Methodical characterization of rice (*Oryza sativa*) bran oil from Pakistan

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**SUMMARY**

**methodical characterization of rice (*Oryza sativa*) bran oil from Pakistan**

The hexane-extracted oil content of four varieties of rice (*Oryza sativa*) viz. Super Kernel, 386, 385 and Basmati, was ranged 14.70-19.10%. Other physical and chemical parameters of the extracted oils were as follow: Iodine value 112.40, 109.80, 105.1 and 103.70; refractive index (40°C) 1.4650, 1.4680, 1.4657 and 1.4660; density (40°C) 0.919, 0.913, 0.909 and 0.911; saponification value 183, 177, 186 and 190; unsaponifiable matter 6.15, 5.60, 4.98 and 5.40% respectivly.

**Tocopherols (α, γ, and δ) in the oils were**: 284.00, 175.12, 180.40, 300.06; 83.40, 98.70, 90.60, 135.74; 196.00, 125.00, 210.0, 276.41; 72.50, 20.00, 39.30 and 64.00 mg/kg, respectively. The content of tocotrienols (α, γ, and δ) in the oils were: 120.30, 106.00, 95.20, 135.74; 196.00, 125.00, 210.0, 276.41; 72.50, 20.00, 39.30 and 64.00 mg/kg, respectively. In the oils studied were found to contain high levels of oleic acid 42.67, 38.59, 40.68 and 36.78% followed by linoleic and palmitic acids 31.58, 33.80, 28.70, 30.51 and 17.00, 14.88, 19.63, 20.00% respectively. The contents of myristic, stearic and arachidic acids were 1.50, 2.02, 4.28, 1.00; 2.64, 2.87, 4.02, 7.48; and 1.28, 3.00, 1.00 respectively. A number of parameters of the investigated rice bran oils indigenous to Pakistan were comparable to those of typical rice bran and some other vegetable oils, reported in the literature. The results of the present analysis as compared with those of different vegetable oils demonstrated rice bran to be a potential oil source and thus could be useful for the establishment of a globalized database of this valuable crop.

**KEY-Words:** Characterization - γ-oryzanol - Oxidative stability - Rice bran – Tocopherols.

**1. INTRODUCTION**

More than 500 million metric tons of rice (*Oryza sativa*) is produced per year worldwide, constituting more than a quarter of all cereal grains. Rice represents a staple food group for many cultures, and has provided the nutritional basis for mankind since antiquity. The people of Asia, South America, much of Africa and portions of Europe, the Near East and North America depend upon rice for daily sustenance (Sugano and Suji, 1997; Sugano and Suji, 1996).

Rice bran is one of the abundant co-products produced in the rice milling industry to yield familiar white rice (Saunders, 1986). Although, it has been recognized as an excellent source of vitamins and minerals, it has been under-utilized as a human food. It has traditionally been used primarily in animal foods. Research conducted during the last two decades has shown that rice bran is a unique complex of naturally occurring antioxidant.
compounds (Moldenhauer et al., 2003). It has been reported to inherently contain a high level of medicinally important antioxidant gamma oryzanol, a natural mixture of ferulic esters (Saunders, 1986), which has professed health benefits including hypolipidamic, growth promotion, development of lean muscles and stimulation of hypothalamus (Seetharamiah and Chandrasekhrar, 1989; Rukmani and Raghuram, 1991). Other physiological effects include decrease of hepatic cholesterol biosynthesis, and plasma cholesterol. Oryzanol has also been accredited for its ability to reduce the cholesterol absorption (Rong et al., 1997).

A number of minor components in rice bran have also been found, through controlled studies with laboratories animals, to exhibit anti-cancer activities. Rice bran protein is unique in its nutritional value, which is much higher than rice endosperm protein or protein from any other cereals or legumes (Tang et al., 2003; Kawamura and Muramoto, 1994). Several clinical studies of cardiovascular health and cholesterol metabolism have been carried out using rice bran oil (Kawamura and Muramoto, 1994). The potential role of rice bran components in bone health is a critical area of research and expands our potential for reducing osteoporosis with functional foods.

The physicochemical properties of the rice kernel, flour, and starch have been studied and reviewed previously (Nakazawa et al., 1984). The composition of rice bran varies depending on the source of the bran, the milling and stabilization techniques used (Malekian et al., 2001; Lloyd et al., 2000) grouped rice bran on the basis of grain size: long, medium, or short grain.

In recent years, rice bran has been reckoned as a potential source of edible oil. (Young-Hee et al., 2002) reported rice bran contained 15-20% of oil, depending upon degree of milling, variety and other agroclimatic factors. Rice bran oil, in its natural state, contains several constituents of potential significance in diet and health. Interest has focused primarily upon gamma-oryzanol, tocotrienols, and tocopherols, all of which demonstrate antioxidant properties (Sugano and Tsuji, 1997; Nicolosi et al., 1997; Westrate and Meiger, 1998). The complete oryzanol group is unique to rice bran oil, but the exact composition of oryzanol depends on the rice cultivars. Significant differences in the levels of tocotrienols and oryzanol from commercially available rice bran oil have been reported by Nicolosi et al. (1997b). Diack and Saska, (1994) described the individual concentrations varied substantially according to the origin of the rice bran.

Saunders (1986) described the composition and potential food uses of rice bran oil. Hemavathy and Prabhakar (1987) studied the lipid composition of three varieties of rice bran from India. Lipids extracted amounted to 21.9-23.0% of the bran dry weight, consisted of 88.1-89.2% neutral lipids, 6.3-7.0% glycolipids and 4.5-4.9% phospholipids. (Sekhon et al., 1997) investigated the functional suitability of commercially milled rice bran for use in different food products. They also studied the effect of blending of commercially available full fat and defatted rice bran from modern multistage rice mills.

In view of growing demand and scientific awareness about the nutritional and functional properties of oils, the quality assessment and composition of non-conventional oilseeds crops is of much concern to cope the existing challenge. Until now, a full characterization and comparison of rice bran oil of different rice cultivars indigenous to subcontinent, and particularly Pakistan has not been reported. In this context, as a part of our preliminary studies (Anwar and Bhanger, 2003) of exploitation of some non-conventional oil seed crops, the present investigation was undertaken to provide information on the detailed analytical characteristics of four varieties of rice bran indigenous to the province of Punjab, Pakistan.

2. MATERIALS AND METHODS

2.1. Product selection

Bran samples of four medium grain Rice varieties; Super kernel, 386, 385 and Basmati were obtained from local rice processing mills in Gujranwala, and Jaranwala, Pakistan. Freshly milled bran samples were collected directly from the milling system in polyethylene bags. These bags were made air tight and stored at 4°C in a cooler.

2.2. Reagents and chemicals

All reagents (analytical and HPLC) used were from E. Merck or Sigma Aldrich. Sterol standards were of Fluka Chemie Gmbh, Sigma-Aldrich (CH-9471, Buchs, Switzerland). Pure standard of \(\gamma\)-Oryzanol was obtained from Sigma Chemical Co. Ltd, Japan. Pure standards of tocopherols \([\Delta\alpha\text{-tocopherol, tocotrienol; } (+)\delta\text{-tocopherol, tocotrienol; } \gamma\text{-tocopherol and tocotrienol}]\), and fatty methyl esters were from Sigma Chemical Co. (St. Louis, MO).

2.3. Extraction of oil

The air-dried bran (500 g) of each batch of four rice varieties was fed to a Soxhelt extractor fitted with a 2-L round-bottom flask and a condenser. The extraction was executed on a water bath for 6 hours with 1.5L of n-hexane. The solvent was distilled off under vacuum in a rotary evaporator. Except for a small quantity (for tocopherol, tocotrienol, \(\gamma\)-Oryzanol
and Rancimat analysis), the recovered oil from different batches was further degummed.

2.4. Analysis of extracted oils

**Physical and chemical parameters of oils.** Determination of iodine value, refractive index, density, peroxide value, saponification value and unsaponifiable matter of extracted oil was carried out by various standards AOCS methods (AOCS, 1992). The color of oils was determined by a Lovibond Tintometer (Tintometer Ltd, Salisbury, U. K) using 1 in. cell. Specific extinctions at 232 and 268 nm were determined using a Hitachi, U-2001, model 121-0032 spectrometer. Samples were diluted with iso-octane to bring the absorbance within limits (0.2-0.8) and \(\varepsilon_{1cm}(\lambda)\) was calculated following the method of IUPAC (IUPAC 1987).

**Determination of \(\gamma\)-Oryzanol.** Analysis of the \(\gamma\)-Oryzanol component from crude oils was performed by HPLC following a previously reported (McBride et al., 1973) reverse phase HPLC method. HPLC unit model Hitachi L-6200, specifications as: column (C18, 150 x 2.1 mm, 5 m), UV / Vis. detector at 330 nm. Flow rate 1.6 mL/min., sample volume 20 µL, and mobile phase was methanol, acetonitrile, dichloromethane, and acetic acid (50: 45: 3: 2 by vol.). The total analysis time was approx. 20 min. and \(\gamma\)-Oryzanol peaks were appeared around 16-18 min of retention times. Pure standard of \(\gamma\)-Oryzanol was used for identification and calibration. A Hitachi Chromatointegrator model D-2500 with a built-in computer program was used for data handling and quantification.

**Determination of tocopherol and tocotrienol content.** Tocopherols and tocotrienols (\(\alpha\),\(\gamma\), and \(\delta\)) analysis was carried out by high-performance liquid chromatography (HPLC) following the method of Hatina and Thompson (Hatina and Thompson 1979) with slight modifications. 0.5 g of oil was accurately weighed and made up to volume with heptane in a 10-mL volumetric flask wrapped in foil to inhibit oxidation. A Hitachi, L-6200 HPLC unit coupled with a Hitachi F-1050 fluorescence detector was used. A 20-µL sample was injected into an analytical column (250 x 4.9 mm) packed with LiChrosorb Si 605 (5 m), which was fitted with a 50 mm x 50 mm (i.d.) guard column with He-Pellosil packing. A mobile phase of dry heptane/ water-saturated heptane / 2-propanol (48.0/49.5/1.5) was used at the rate of 1.5 mL/min. Detection was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Pure standards of \(\alpha\),\(\gamma\), and \(\delta\) tocopherols and their corresponding tocotrienols were used for identification and calibration. A Hitachi Chromatointegrator model D-2500 with a built-in computer program for data handling was used for the quantification.

**Oxidative stability.** An automated Metrohm Rancimat Model 679, capable of operating over a temperature range of 50-200°C (Metrohm Application Bulletin 1993), was used for the determination of induction periods (IP) of the degummed and non-degummed oils. Testing was carried out at 120 \(\pm\) 0.1 °C and oxidative stability was measured following the procedure described elsewhere (Anwar et al., 2003). Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. IP of the samples were automatically recorded and corresponded to the break point of the plotted curves.

**Determination of sterol composition.** The determination of sterols was made following the Official method of the Association of Official Analytical Chemists (AOAC, 1984). Analysis was carried out on a Perkin Elmer gas chromatograph model 8700, equipped with methylphenyl polysiloxanes coated capillary column OV-17 (30m x 0.25mm, 0.20µm film thickness) and a flame ionization detector (FID). The column was isothermally operated at temperature of 260 °C. Injector and FID temperature were set at 275 and 290 °C, respectively. Extra pure \(N_2\) at a flow rate of 3.5 mL min\(^{-1}\) was used as a carrier gas. The internal standard used was 5-\(\alpha\)-cholestane, and identification and quantification of unknown sterol components was made using a pure mixture of sterol standards.

**Determination of fatty acid composition.** Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on a SHIMADZU gas chromatograph model 17-A fitted with a methyl linoserase coated (film thickness = 0.20 µm), polar capillary column SP™-2330 (30 m’ 0.32 mm), and a flame ionization detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 4.0 mL/min. Other conditions were as follow: initial oven temperature 140°C; ramp rate 4 °C/min; final temperature 230°C; injector temperature, 250°C; detector temperature, 260°C; and temperature hold, 2 min. before the run and 4 min. after the run. A sample volume of 1.0 µL was injected. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters. A Chromatographic Station (CSW32) data-handling program did all of the evaluation and quantification. The fatty acid composition was reported as a relative percentage of the total peak area.

3. RESULTS AND DISCUSSION

The data for the analysis of hexane-extracted oils from four rice bran varieties, together, with literature values have been summarized in Tables 1-7. Values for the present analysis are given as mean SD of
three rice bran oils of each variety, prepared separately and analyzed individually in triplicate. The hexane extracted oil content of different varieties of rice bran was ranged from 14.70 to 19.10 % (Table 1). Rice bran varieties 385 and Super Kernel were found to be the highest and lowest in their oil content i.e., 19.10%, 14.70 % respectively. This variation in the yield of oil from different varieties of rice bran might be attributed to the diversity in natural soil texture of their derivation and other man-made cumulative effects.

The range of rice bran oil content (14.70-19.10 %) in the present work was quite comparable to that reported from Korea (Lee et al., 2002) and Bangladesh (Absar et al., 1998). However, Super Kernel variety of rice bran from Pakistan was found to be lower in oil content to that reported from Bangladesh. Such minor variation in the oil content within the countries may be attributed to the possible change in environmental and geological conditions of the regions (Ibrahim et al., 1994). This range of oil content determined in the present analysis of different varieties of rice bran was generally found to exceed those of cottonseed (10 -12%), indigenous to Pakistan (http://www.dawn.com/2001/10/15/ebr14.htm), and mango kernel grown in India (Rossell, 1991). The content of oil was somewhat comparable to that of cottonseed (15.0-24 %) grown in United States.
Brazil, China and some other Asian and European countries (Rossell, 1991a).

The results of various physical and chemical parameters are given in Table 2. The values reported for iodine, refractive index, density, saponification value and unsaponifiable matter of different rice bran oils were in close agreement with those reported in the literature (Rossell, 1991b). The crude rice bran oil of variety 386 was superior in color measurement (4.5R+25.00 Y) than crude rice bran oil reported from India (Bhattacharyya and Bhattacharyya, 1989), While super kernel variety was found with highest color index (7.50R +30.00Y) followed by 385 (7.00R+30.50Y) and Basmati (5.00R+28.00Y). The intensity of color of vegetable oils depends mainly upon the presence of various pigments such as chlorophyll, which are effectively removed during the degumming, refining and bleaching step of oil processing. The vegetable oils with minimum value of color index are more suitable for edible and domestic purposes.

Iodine values of Super kernel (112.40 g of 1/100g of oil), 386 (109.80 g of 1/100g of oil), 385 (105.10 g of 1/100g of oil) and Basmati (103.70 g of 1/100g of oil) were comparable with that of rice bran oil reported in the literature (Rossell, 1991b) and also lies with in the range of corn, cottonseed, mustard seed, high erucic acid and sesame seed oils (Rossell, 1991b). The values of refractive indexes (1.4650-1.4680) of the investigated oils were

<table>
<thead>
<tr>
<th>Determination</th>
<th>Super Kernel</th>
<th>386</th>
<th>385</th>
<th>Basmati</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε₁cm (λ232)</td>
<td>2.00 ± 0.03</td>
<td>1.85 ± 0.04</td>
<td>3.00 ± 0.03</td>
<td>2.45 ± 0.05</td>
<td>present work</td>
</tr>
<tr>
<td>ε₁cm (λ270)</td>
<td>0.89 ± 0.01</td>
<td>1.30 ± 0.02</td>
<td>1.00 ± 0.01</td>
<td>0.77 ± 0.01</td>
<td>present work</td>
</tr>
<tr>
<td>Peroxide value (meq/kg of oil)</td>
<td>1.5 ± 0.05</td>
<td>3.00 ± 0.03</td>
<td>2.50 ± 0.04</td>
<td>2.4 ± 0.03</td>
<td>present work</td>
</tr>
<tr>
<td>Para anisidine value</td>
<td>3.30 ± 0.10</td>
<td>4.00 ± 0.10</td>
<td>2.94 ± 12.0</td>
<td>3.08 ± 0.12</td>
<td>present work</td>
</tr>
<tr>
<td>Oxidative Stability&lt;sub&gt;ndmo&lt;/sub&gt;</td>
<td>6.81 ± 0.20</td>
<td>5.99 ± 0.15</td>
<td>6.39 ± 0.30</td>
<td>7.40 ± 0.25</td>
<td>present work</td>
</tr>
<tr>
<td>(Rancimat method (hours))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD for three rice bran oils of each variety, analyzed individually in triplicate.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Amount of γ- Oryzanol (µg/g)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super Kernel</td>
<td>802.05 ± 15.21</td>
<td>present work</td>
</tr>
<tr>
<td>386</td>
<td>639.11 ± 20.48</td>
<td>present work</td>
</tr>
<tr>
<td>385</td>
<td>415.12 ± 7.07</td>
<td>present work</td>
</tr>
<tr>
<td>Basmati</td>
<td>778.54 ± 18.29</td>
<td>present work</td>
</tr>
</tbody>
</table>

Values are mean ± SD for three rice bran oils of each variety, analyzed individually in triplicate.
fairly comparable to those of cocoa butter, shea nut, maize, Indian-illipe, kapok, pumpkin, high erucic acid, sesame and tomato seed oils (Rossell, 1991b). The range of saponification values (177-190 mg of KOH/g of oil) and unsaponifiable matter (4.98-6.15 %), were in close agreement with those of rice bran and shea nut oil reported in the literature (Rossell, 1991b). These variations in the physical and chemical characteristics of the investigated oils of different rice cultivars may be attributed to the source and milling process of rice polishing industry.

<table>
<thead>
<tr>
<th>Determination</th>
<th>Super Kernel</th>
<th>386</th>
<th>385</th>
<th>Basmati</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>284.00 ± 4.60</td>
<td>175.12 ± 3.00</td>
<td>180.42 ± 4.00</td>
<td>300.06 ± 3.05</td>
<td>present work</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>83.40 ± 2.30</td>
<td>98.70 ± 2.00</td>
<td>120.70 ± 3.50</td>
<td>90.60 ± 3.50</td>
<td>present work</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>75.16 ± 2.25</td>
<td>57.20 ± 1.97</td>
<td>39.32 ± 2.15</td>
<td>83.00 ± 3.10</td>
<td>present work</td>
</tr>
<tr>
<td>α-tocotrienol</td>
<td>120.30 ± 3.00</td>
<td>106.00 ± 2.10</td>
<td>95.20 ± 1.85</td>
<td>135.74 ± 2.80</td>
<td>present work</td>
</tr>
<tr>
<td>γ-tocotrienol</td>
<td>196.00 ± 2.00</td>
<td>125.00 ± 3.00</td>
<td>210.00 ± 3.28</td>
<td>276.41 ± 1.90</td>
<td>present work</td>
</tr>
<tr>
<td>δ-tocotrienol</td>
<td>72.50 ± 1.50</td>
<td>20.00 ± 1.00</td>
<td>39.30 ± 0.5</td>
<td>64.00 ± 0.47</td>
<td>present work</td>
</tr>
</tbody>
</table>

Values are mean ± SD for three rice bran oils of each variety, analyzed individually in triplicate

<table>
<thead>
<tr>
<th>Determination</th>
<th>Super Kernel</th>
<th>386</th>
<th>385</th>
<th>Basmati</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>campesterol</td>
<td>10.10 ± 0.30</td>
<td>15.50 ± 0.42</td>
<td>19.20 ± 0.40</td>
<td>12.30 ± 0.30</td>
<td>present work</td>
</tr>
<tr>
<td>Δ7, campestanol</td>
<td>-----nd</td>
<td>0.50 ± 0.10</td>
<td>1.40 ± 0.05</td>
<td>-----nd</td>
<td>present work</td>
</tr>
<tr>
<td>stigmasterol</td>
<td>19.28 ± 0.50</td>
<td>15.90 ± 0.27</td>
<td>14.00 ± 0.25</td>
<td>17.12 ± 0.37</td>
<td>present work</td>
</tr>
<tr>
<td>stigmastanol</td>
<td>0.75 ± 0.09</td>
<td>1.00 ± 0.10</td>
<td>-----nd</td>
<td>0.90 ± 0.06</td>
<td>present work</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>58.20 ± 0.62</td>
<td>51.10 ± 0.50</td>
<td>49.30 ± 1.00</td>
<td>54.80 ± 0.40</td>
<td>present work</td>
</tr>
<tr>
<td>Δ7, avenasterol</td>
<td>1.20 ± 0.10</td>
<td>1.95 ± 0.12</td>
<td>0.95 ± 0.06</td>
<td>2.70 ± 0.05</td>
<td>present work</td>
</tr>
<tr>
<td>Δ5, avenasterol</td>
<td>8.14 ± 0.15</td>
<td>12.00 ± 0.20</td>
<td>13.05 ± 0.43</td>
<td>10.15 ± 0.20</td>
<td>present work</td>
</tr>
<tr>
<td>28, isoavenasterol</td>
<td>1.0 ± 0.07</td>
<td>0.85 ± 0.10</td>
<td>0.50 ± 0.06</td>
<td>-----nd</td>
<td>present work</td>
</tr>
</tbody>
</table>

Values are means ± SD of three rice bran oils of each variety, analyzed individually in triplicate

-----nd not detected
The investigated rice bran oils also exhibited very good oxidative state as indicated by the determinations shown in Table 3. The specific extinctions at 232, 270 nm, which revealed the oxidative deterioration and purity of the oils (Yoon et al., 1985), were quite low which reflected a high stability of the oils. The induction periods (Rancimat; 20 L/h, 120 °C), which is a characteristic of the oxidative stability of the oil and fats (Anwar et al., 2003), of the non-degummed Super kernel, 386, 385 and Basmati rice bran oils were 6.81, .99, 6.39 and 7.40 hrs respectively, indicating a high resistance to oxidation and stability.

The peroxide value (meq/kg of oil) and \( \text{p-anisidine value} \), which measure hydroperoxides and aldehydic secondary oxidation products of the oils (McGinely, 1991), were quite low i.e., Super kernel (1.50, 3.30), 386 (3.00, 4.00), 385 (2.50, 2.94) and Basmati (2.40, 3.08) respectively. The values of PV, \( \text{p-anisidine} \) and induction periods as determined in the present analysis could not be compared as there are no previously reported data of rice bran oil to compare the results with our present work. A high oxidative stability of rice bran oil, exhibited in the present analysis, compared with those of conventional vegetable oils (Anwar et al., 2003), could be attributed to a significantly higher level of C18:1, which is less prone to oxidation than polyenoics (Anwar et al., 2003). Moreover, a high resistance to oxidation of rice bran oil might be explained due to the presence of high content of \( \gamma \)-oryzanol and \( \alpha-, \gamma- \) and \( \delta- \)tocopherols.

Rice bran oils were also found to contain significantly high amount of \( \gamma \)-Oryzanol (Table 4). This substance is composed of several kinds of ferulic acids and has an effect similar to that of Vitamin E in accelerating human growth, facilitating blood circulation and stimulating hormonal secretion (Rong et al., 1997). Besides beneficially influencing the lipid profiles, oryzanol is also known to have anti-itching, anti-dandruff and anti-aging properties. It is also effective in treating a broad range of gastrointestinal disorders including stress - induced gastric and duodenal ulcers (Rukmani and Raghuram, 1991; Rukmani, 1995).

In some of the cases, there was strong correlation between the contents of \( \gamma \)-Oryzanol and oil of the rice brans. Rice bran (Super kernel) having highest content of gamma Oryzanol (802.01ug/gm) was quite lower in its oil content 14.70%. Whereas, rice bran variety 385, which contained lowest gamma Oryzanol 415.12 ug/gm, was highest in its oil content i.e. 19.10%. This indicates that \( \gamma \)-Oryzanol is a major component of crude oil of Super kernel but only minor one; in 385 rice bran oil and thus it may affect the oil concentration. However, there was lack of strong correlation in other two varieties. A similar assessment has also been made in Thailand for the determination of \( \gamma \)-Oryzanol in purple rice grains.

Table 5 shows the content of different tocopherols and tocotrienols in the non-degummed (crude) rice bran oils as determined by HPLC. The level of \( \alpha- \)tocopherol, \( \gamma- \)tocopherol and \( \delta- \)tocopherol in the Super kernel, 386, 385, basmati oils was 284.00, 83.40, 75.16 mg/kg; 175.12, 98.70, 57.20 mg/kg; 180.42, 120.70, 39.30 mg/kg and 300.06, 90.60, 83.00 mg/kg respectively. The content of tocopherol ranged 175.12-300.06 mg/kg, which has greatest vitamin E potency (Rossell, 1991b), in the investigated oils was higher than the values reported in the literature.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Super Kernel</th>
<th>386</th>
<th>385</th>
<th>Basmati</th>
<th>Literature (Rossell, 1991)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1.50 ± 0.10</td>
<td>2.02 ± 0.09</td>
<td>4.28 ± 0.10</td>
<td>1.0 ± 0.05</td>
<td>0.50</td>
</tr>
<tr>
<td>C16:0</td>
<td>17.00 ± 0.22</td>
<td>14.88 ± 0.16</td>
<td>19.63 ± 0.18</td>
<td>20.00 ± 0.21</td>
<td>16.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.64 ± 0.10</td>
<td>2.87 ± 0.04</td>
<td>4.02 ± 0.21</td>
<td>7.48 ± 0.35</td>
<td>1.50</td>
</tr>
<tr>
<td>C18:1</td>
<td>42.67 ± 0.45</td>
<td>38.59 ± 0.53</td>
<td>40.68 ± 0.50</td>
<td>36.78 ± 0.60</td>
<td>42.5</td>
</tr>
<tr>
<td>C18:2</td>
<td>31.58 ± 0.58</td>
<td>33.80 ± 0.50</td>
<td>28.70 ± 0.51</td>
<td>30.51 ± 0.53</td>
<td>35.50</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.50 ± 0.04</td>
<td>1.00 ± 0.09</td>
<td>tr</td>
<td>2.43 ± 0.04</td>
<td>1.00</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.28 ± 0.09</td>
<td>3.00 ± 0.06</td>
<td>1.00 ± 0.04</td>
<td>1.00 ± 0.09</td>
<td>0.50</td>
</tr>
<tr>
<td>C20:1</td>
<td>1.00 ± 0.06</td>
<td>2.90 ± 0.07</td>
<td>tr</td>
<td>tr</td>
<td>0.50</td>
</tr>
<tr>
<td>C22:0</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.50</td>
</tr>
<tr>
<td>C24:0</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Values are means ± SD for three rice bran oils of each variety, analyzed individually in triplicate. --- nd not detected.
for palm kernels, coconut and palm oils, while it was
well in line to those reported for soybean, ground nut,
high and low erucic acid rapeseed oils (Rossell,
1991b). The level of tocopherol (83.40-120.70 mg/kg) was
significantly higher to those of coconut, palm
and sunflower oils, whereas lower to those of
cottonseed, soybean, maize and high erucic acid
rapeseed oils (Rossell, 1991b). The concentration of
tocopherol ranged 39.32 - 83.00 mg/kg, which has a
greater antioxidative activity than either γ or
α-tocopherol (Tsaknis, 1999), in the rice bran oils
was comparable to maize oil, whereas, it was
appreciably higher to those of palm kernel, coconut,
cotton seed ground nut, palm sunflower, high and
low erucic acid rapeseed oils (Rossell, 1991), and
thus, would be expected to contribute good oxidative
stability and protection to the oils during storage and
processing.

The contents of α-, γ- and δ-tocotrienols that have
been claimed to give protection against heart attack
due to their anti-thrombotic properties and have
greater antioxidant potential than the tocopherols
(Rukmani, 1995; Rossell, 1991) in the investigated
rice bran oils was ranged 95.20-135.74,
125,000-276.41 and 20.00-72.50 mg/kg respectively.
The contents of isomeric trienols determined in the
present analysis were mainly higher than those of
conventional vegetable oils (Rossell, 1991b). However,
the amount of γ-tocotrienol was found to be lower than
palm oil and comparable to that of maize oil.
The content of δ-tocotrienol was significantly higher to those
of common vegetable oils and comparable to that of
palm oil (Rossell, 1991b). As with many of the other
traits, there were no previously reported data on the
tocopherol and tocotrienol contents of rice bran oil to
compare with our present findings.

The composition of different sterols of rice bran
oils as determined by GLC, is shown in Table 6. The
sterol fractions of Super kernel, 386, 385, basmati rice
bran oils mainly consisted of campesterol (10.10,
15.50, 19.20, 12.30 %), stigmasterol (19.28, 15.90,
14.00, 17.12 %), β-sitosterol (58.20, 51.10, 49.30,
54.80 %), and δ5-avenasterol (8.14, 12.00, 13.05,
10.15 %) together with minute amounts (< 3.00 %) of
28,isoavenasterol, δ5-avenasterol, stigmasterol and
δ5-campestanol.

The contents of major sterols i.e., campesterol
(10.10-19.20%), Stigmasterol (14.00-19.28 %)
, δ5-avenasterol (8.14-13.05%) and β-sitosterol
(49.30-58.20%) of the investigated rice bran oils was
quite comparable with the values for ground nut,
soybean, ground nut and soybean, ground nut,
rapeseed oils respectively, whereas, significantly
varied to those of common vegetable oils (Rossell,
1991b). There were no previously reported data on the
sterol contents of rice bran oil to compare with
our present findings. Regional and cultivars
variations for the distribution of campesterol,
stigmasterol, β-sitosterol, Δ5-avenasterol and
clerosterol have already been reported in the
literature (Norman, 1979).

Table 7 shows the fatty acid (FA) composition of
different rice bran oils. The content of total saturated
fatty acids (SFA): myristic (C 14:0), palmitic (C 16:0),
stearic (C 18:0) and arachidic (C 20:0) acids, in the Super
kernel, 386, 385 and Basmati oils were 22.42, 22.77,
28.93 and 29.48 % respectively. The level of SFA
contents of Super Kernel and 386 rice bran oils as
investigated in the present analysis was comparable
to the values of rice bran, palm kernel, cottonseed
and avocado (fruit coat) oils reported in the literature
(Rossell, 1991b) but varied to those of other
commonly grown and typical vegetable oils (Rossell,
1991b).

The investigated oils were found to contain a high
level of monounsaturated fatty acids (MUFA) up to
43.67, 41.49, 40.68 and 36.78% respectively. The
level of oleic acid (C 18:1 9), which, accounted for
42.67, 38.59, 40.68 and 36.78% of the total fatty
acids was rather comparable to those of rice bran,
palm and allanblackia oils reported in the literature
(Rossell, 1991b). The content of linoleic acid (C 18:2
ω-6) in the investigated varieties was 31.58, 33.80,
28.70 and 30.51% respectively. The content of
linoleic acid (C 18:2 ω-3) in Super kernel, 386, and
Basmati variety was found to be 1.50, 1.00 and 2.43
% respectively. The amount of C 18:2 ω-6 in the present
analysis of Super kernel, 386, and Basmati varieties
of rice bran oil was in close agreement to that of
literature value (Rossell, 1991b) and found to exceed
those of palm kernel, coconut, palm, high and low
erucic acid oils (Rossell, 1991b).

The concentration of major fatty acids, C 18:2, C 18:1,
C 16:0, C 16:9 of the investigated oils were in close
agreement with that reported by Hemavathy and
Prabhakar (1987), for the rice bran oils indigenous in
India. The amount of C 16:0 in 385 and 386 varieties,
C 18:2 in 385 and Basmati varied to some extent from
those reported in the literature (Hemavathy and
Prabhakar 1987; Saunders, 1986).

The fatty acid composition of the investigated oils
was quite similar in contents of C 18:2 with those of rice
bran oil (Lee et al., 2002) indigenous to Korea.
However, amounts of C 18:2 were varied to some
extent. The present fatty acid analysis of the rice bran
oil produced from Pakistan showed that oleic acid
was the predominant fatty acid followed by linoleic
(ω-6) and palmitic acid.

The Japans Ministry of health and welfare
suggests fatty acid ratio of saturated/
monounsaturated/polyunsaturated for the healthy
edible oils as 1:1.5:1. The fatty acid composition of
the investigated rice bran oils from Pakistan falls in
the recommendations and contains high ratio of
monounsaturated to saturated fatty acids. As the rice
bran oil is unique in its fatty acid composition and
thought to be one of the highest quality of vegetable oil. As with humans, a number of fatty acids are required in animal diets. It appears that rice bran oil is roughly equal to other vegetable oils in supplying fatty acid requirements but unique in its fatty acid composition. In consideration of this and above properties, rice bran oil has much to offer in caring for nutritional and health needs of the pets and other animals in addition to human beings.

4. CONCLUSION

Pakistan is among the four major rice-exporting countries of the world and ranks 13th among 112 rice producing countries. Rice exports now have been raised from 1.3 million tons (1983) to 2.95 million tons in 2001, thus contributing to the generation of a huge quantity of rice bran as an agricultural waste which could be utilized for the production of useful oil. It was concluded from the results of the present investigations that rice bran oil might be an acceptable substitute for some high oleic-oils in the dietary fats as well as it could be employed for developing nutritionally balanced, high-stability blended formulations with other high-linoleic oils.

5. REFERENCE


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Recibido: Septiembre 2004
Aceptado: Enero 2005